Role of the area postrema in angiotensin II modulation of baroreflex control of heart rate in conscious mice

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Submitted 9 September 2002; accepted in final form 14 November 2002

Xue, Baojian, Hope Gole, Jaya Pamidimukkala, and Meredith Hay. Role of the area postrema in angiotensin II modulation of baroreflex control of heart rate in conscious mice. Am J Physiol Heart Circ Physiol 284: H1003–H1007, 2003; 10.1152/ajpheart.00793.2002.—This study reports the effects of angiotensin II (ANG II), arginine vasopressin (AVP), phenylephrine (PE), and sodium nitroprusside (SNP) on baroreflex control of heart rate in the presence and absence of the area postrema (AP) in conscious mice. In intact, sham-lesioned mice, baroreflex-induced decreases in heart rate due to increases in arterial pressure with intravenous infusions of ANG II were significantly less than those observed with similar increases in arterial pressure with PE (slope: −3.0 ± 0.9 vs. −8.1 ± 1.5 beats·min⁻¹·mmHg⁻¹). Baroreflex-induced decreases in heart rate due to increases in arterial pressure with intravenous infusions of AVP were the same as those observed with PE in sham animals (slope: −5.8 ± 0.7 vs. −8.1 ± 1.5 beats·min⁻¹·mmHg⁻¹). After the AP was lesioned, the slope of baroreflex inhibition of heart rate was the same whether pressure was increased with ANG II, AVP, or PE. The slope of the baroreflex-induced increases in heart rate due to decreases in arterial blood pressure with SNP were the same in sham- and AP-lesioned animals. These results indicate that, similar to other species, in mice the ability of ANG II to acutely reset baroreflex control of heart rate is dependent on an intact AP.

METHODS

All experiments were carried out in c57 strain wild-type male mice. These mice were a gift from Dr. Dennis Lubahn (University of Missouri, Columbia, MO). The experiment was composed of the experimental group of AP-lesioned mice and the control group of AP sham-lesioned mice.

AP lesion. Mice (6–7 mo old) were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) supplemented with isofluorane when necessary. The AP was exposed by opening the atlantooccipital membrane and was removed using vacuum aspiration. The AP sham-lesioned mice underwent the same procedure except that the AP was not removed. At the end of the experiment, the animals were euthanized and perfused. The accuracy of the AP lesions and AP sham lesions was verified histologically.

Instrumentation. The mice were instrumented with chronic arterial and venous catheters 4 mo postAP lesion surgery. Animals were anesthetized as described above. Arterial and venous catheters constructed of Micro-Renathane tubing (MRE-025, 0.64 mm outer diameter (OD), 0.30 mm internal diameter (ID)) were percutaneously inserted into the right carotid artery and the right jugular vein. Chronic arterial and venous catheters were constructed of 0.035” Micro-Renathane tubing and were tunneled subcutaneously to the neck where they were fixed with sutures. The chronic arterial and venous catheters were used to measure central arterial pressure and to take blood samples for determination of circulating vasoactive peptides such as ANG II and AVP. The animals were trained to wear adapted nose cones on a chronic basis for measurement of ventilation, and for administration of intravenous drugs. The animals were also trained to wear a head support for measurement of arterial blood pressure while anesthesia was induced with isoflurane (0.5–3%).

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inner diameter (ID), Braintree Scientific were chronically implanted into the femoral artery and vein for the measurement of arterial pressure and administration of drugs, respectively. The free ends of these catheters were tunneled subcutaneously and exteriorized at the back of the neck. Catheters were then threaded through polyethylene tubing (2.80 mm OD, 1.77 mm ID, Becton-Dickinson). The tubing was secured with sutures. The experiments were carried out in conscious, freely moving mice 4–5 days after surgery.

**Evaluation of cardiac baroreflexes.** Arterial blood pressure was recorded with a MLT0698 disposable blood pressure transducer. Heart rate and mean arterial pressure (MAP) were derived by data-acquisition software (Powerlab) using the arterial pressure pulse. Cardiac baroreflexes were evoked by increasing arterial pressures with ramp infusions of either phenylephrine (PE; 1.0 mg/ml), ANG II (100 μg/ml), or AVP (25 μM) and by lowering blood pressure with sodium nitroprusside (SNP; 1.0 mg/ml). Infusion rates (0.003 ml/min) were monitored such that blood pressure was increased or decreased 30–40 mmHg over a 30- to 45-s period. The drug order was randomized, and 45–60 min were allowed between each curve.

**Statistical analysis.** Data are presented as means ± SE. Statistical significance was determined using a paired t-test or two-way ANOVA where needed. To analyze baroreflex responses, the absolute value of heart rate for each dose of ANG II, AVP, PE, or SNP was plotted against the corresponding value of MAP for each mouse, and the data were subjected to linear regression analysis. A value of $P < 0.05$ was considered to be statistically significant.

Fig. 1. Resting mean arterial blood pressure (MAP; in mmHg) and heart rate (HR; in beats/min) in male mice with intact (sham; $n = 8$) or lesioned area postrema (APX; $n = 10$). Resting values for MAP and HR were not significantly different between sham and APX mice ($P > 0.05$).

**RESULTS**

Resting values for blood pressure and heart rate were not significantly different between sham ($n = 8$, 122 ± 2 mmHg and 691 ± 20 beats/min) and lesioned animals ($n = 10$, 120 ± 4 mmHg and 679 ± 16 beats/min) (Fig. 1).

The linear regression analysis of the heart rate baroreflex responses to intravenous infusions of ANG II and PE in sham- and APX-lesioned mice are shown in Fig. 2 and Table 1. In sham-lesioned animals, the slope of the line for the ANG II-induced heart rate response was $-3.0 \pm 0.9$ beats·min$^{-1}$·mmHg$^{-1}$ and was significantly less than that observed with PE ($-8.1 \pm 1.5$ beats·min$^{-1}$·mmHg$^{-1}$). In APX-lesioned animals, the slope of the line for the ANG II-induced heart rate ($-7.2 \pm 1.0$ beats·min$^{-1}$·mmHg$^{-1}$) was the same as that observed with PE ($-7.7 \pm 1.0$ beats·min$^{-1}$·mmHg$^{-1}$).

Figure 3 illustrates the linear regression analysis of the heart rate baroreflex responses to intravenous AVP (Fig. 3 and Table 1) in sham- and APX-lesioned animals. In sham-lesioned animals, the slope of the line for the AVP-induced heart rate response was $-5.8 \pm 0.7$ beats·min$^{-1}$·mmHg$^{-1}$, which was similar to that observed in the APX-lesioned animals ($-6.1 \pm 1.0$ beats·min$^{-1}$·mmHg$^{-1}$).

The baroreflex responses to SNP infusions are summarized in Fig. 4 and Table 1. The slope of the line for
the heart rate response to SNP was the same in sham- and AP-lesioned animals.

Figure 5 shows photomicrographs of the dorsal medulla at the level of the obex in representative sham-lesioned (A) and AP-lesioned mice (B). The lesion extended throughout the AP and caused minimal damage to surrounding structures.

**DISCUSSION**

In the present study, we evaluated the effects of AP lesion on baroreflex control of heart rate during increases in arterial pressure with PE, ANG II, and AVP or decreases in arterial pressure with SNP. We found that in conscious mice 1) ANG II-induced baroreflex inhibition of heart rate was significantly blunted relative to those observed with PE, 2) AVP-induced baroreflex inhibition of heart rate was similar to those observed with PE in both the sham and lesioned mice, 3) lesioning of the AP had no effect on the PE-induced inhibition of heart rate, and 4) lesioning of the AP facilitated ANG II-induced baroreflex control of heart rate and normalized the slope of the ANG II-induced arterial pressure-heart rate curve to that seen with PE. These results suggest that the AP is involved in the central actions of ANG II on baroreflex control of heart rate in mice.

The AP is a circumventricular organ localized in the hindbrain. Not only is the AP directly accessible to circulating peptides and hormones, it is anatomically and functionally well situated to have a direct effect on central neurons involved in cardiovascular regulation (15). The physiological evidence for the participation of the AP in central reflex function and cardiovascular control is, in part, based on the action of the circulating peptides ANG II and AVP (17, 27, 40). Both of these peptides have been shown to modulate cardiovascular function through interactions at the level of the AP. Ablation studies in rats, rabbits, and dogs have demonstrated that the central effect of ANG II on blood pressure regulation and neurogenic hypertension is dependent on the AP (3, 11, 18, 29). Additionally, an intact AP is required for ANG II to acutely modulate the arterial baroreflex control of heart rate. In this case, exogenous and endogenous ANG II shifts the baroreflex heart rate response to a higher pressure (20, 29, 30). Thus the decrease in heart rate with a given increase in pressure with ANG II is significantly less than that produced with PE. This difference is normalized after ablation of the AP. In the present study, our results confirm and extend these previous observations in conscious mice.

Like ANG II, a number of studies have also established a role for the AP in the enhancement of baroreceptor-mediated bradycardia and sympathoinhibition by systemically administered AVP. The action of circulating AVP to augment baroreflex responses was abolished by prior lesion of the AP in rabbits, dogs, and rats (1, 31, 40). A recent receptor binding study by Tribollet et al. (38) has shown the high expression of V1 receptors in the murine AP. Thus it could be speculated that AVP may have similar actions in mice. To our surprise and contrary to what might have been expected, we found that the AVP-induced baroreflex inhibition of heart rate was similar to that observed with PE in both the sham and lesioned mice. The explanation for the discrepancies seen in the mice is not readily apparent, although species differences may explain the divergent findings.

In most mammals, resting heart rates are predominantly under parasympathetic control. In mice, blockade of parasympathetic tone in general results in a minimal increase in heart rates, an indication of a low parasympathetic drive under resting conditions (13). It

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**Table 1. Slopes of baroreflex responses to PE, ANG II, AVP, and SNP in male mice with an intact or lesioned area postrema**

<table>
<thead>
<tr>
<th>Group</th>
<th>PE</th>
<th>ANG II</th>
<th>AVP</th>
<th>SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>-7.7 ± 1.0</td>
<td>-7.2 ± 1.0</td>
<td>-6.1 ± 1.0</td>
<td>-1.3 ± 0.4</td>
</tr>
<tr>
<td>APX</td>
<td>-8.1 ± 1.5</td>
<td>-3.0 ± 0.9*</td>
<td>-5.8 ± 0.7</td>
<td>-1.0 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE (in beats·min⁻¹·mmHg⁻¹); n = 10 male mice with a lesioned area postrema (APX group) and 8 male mice with an intact area postrema (sham group). PE, phenylephrine; AVP, arginine vasopressin; SNP, sodium nitroprusside. *Significantly smaller compared with PE (P < 0.05).
has also been suggested that the high resting heart rates in mice are a result of a greater sympathetic drive (26). Consequently, in the present study, the robust bradycardic responses to PE could be either due to increases in the parasympathetic drive or a withdrawal of the sympathetic drive to the heart. However, there are several studies in the literature that suggest that, in mice, vagal innervation accounts for more of the total range of heart rate changes than sympathetic innervation (19, 39). This is not unlike the autonomic regulation of heart rate in rats, except in mice the baroreflexes operate around an even higher set point.

In summary, in mice, ANG II-induced baroreflex inhibition of heart rate was significantly blunted relative to that observed with PE. Lesioning of the AP facilitates ANG II-induced baroreflex control of heart rate and normalized the slope of the ANG II-induced arterial pressure-heart rate curve to that seen with PE. These results suggest that the AP is involved in the central actions of ANG II on baroreflex control of heart rate in mice.

This study was supported by National Heart, Lung, and Blood Institute Grant HL-62261.

REFERENCES


